membrane-embedded respiratory complex cyt bc1 [2]. The latter model system that is of greater interest comprises the entire cyt bc1 dimer of the purple photosynthetic bacterium Rhodobacter capsulatus embedded in a lipid bilayer, whose lipid composition mimics that of the inner mitochondrial membrane. Intriguingly CLs were observed to diffuse spontaneously to the dimer interface and to the immediate vicinity of the catalytic Qi-sites [2]. This observation is in agreement with experimental data, as CLs are indeed located close to the Qi-sites in several X-ray crystal structures of the complex. Importantly, our observations support the proposed role of CL in delivering protons for the non-reduced substrate forms in the active site.
In ongoing work that we discuss here we focus more specifically on the roles of individual components of the proposed proton uptake pathway (CL, water, and individual protein residues) and on the atom-level reaction mechanism in the binding pocket. To this end, further MD simulations, QM calculations, and cite-directed mutagenesis experiments were employed. We also discuss whether there is a plausible pathway for substrate movement between the active sites through the lipid-filled insides of the complex, and the role of oxidative stress in cyt bc1 behavior.
References
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2602-Pos Board B294
Fluorescence Correlation Spectroscopy Reveals Additional Information on Protein Insertions into Phospholipid Monolayers
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The generation of curvature in phospholipid membranes is a crucial step in vesicle and tube formation. A variety of proteins in eukaryotic cells are able to induce such curved membrane structures and produce coated transport vesicles or tubular carriers. Membrane curvature can be generated in different ways. Sar1, which is a small GTPase of the RAS superfamily and a part of the coat protein complex II (COPII), and the N-BAR dimer of amphiphysin II, which is involved in clathrin-coated vesicle formation, both have amphipathic α-helices, which insert only into the proximal leaflet of a phospholipid membrane. Phospholipid monolayers are therefore convenient model systems for analyzing the protein-lipid interactions.
While a typical Langmuir film balance setup allows to observe the interaction of proteins with phospholipid monolayers (by changing the surface pressure or the surface area),the determination of protein concentrations and diffusion properties is not possible.
To overcome these limitations, we combine a Langmuir film balance with a confocal fluorescence correlation spectroscopy setup for optical detection. This setup, which was described for the first time by Gudmand et.al (Biophys. J., 2009, 96, 4598-4609), allows FCS measurements in a monolayer system.
Here we show some basic interaction studies of Sar1 and N-BAR with phospholipid monolayers at different surface pressures. In all cases, FCS measurements were carried out to obtain additional information on the protein.

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Membrane Phosphoinositide Turnover by Voltage Sensing Phosphatases
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The PI levels can be controlled by the state of dynamic equilibrium between lipid kinase-induced phosphorylation and lipid phosphatase-induced dephosphorylation. The voltage sensing phosphatases (VSPs) gain enzymatic activity upon membrane depolarization so they can trigger temporary imbalance of PIs by electrophysiological method. Recent studies reported that VSPs have both 3- and 5- phosphatase activities upon membrane depolarization. However, its enzymatic characteristics and mechanism are still unclear. Here we examined the functional role of Dr-VSP and Ci-VSP fused with PTEN (Ci-VSPTEN) in the PI turnover through Fluorescence Resonance Energy Transfer (FRET) using Pleckstrin Homology (PH) domains. VSP-induced turnover of diverse PIs, such as $PI(3,4)P_2$, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ showed different kinetics in degradation and resynthesis. When the Dr-VSP expressing cells were given with a 120 mV/3 s of depolarization, the level of $PI(4,5)P_2$, indicated by PH(PLCδ) FRET fairs, decreased rapidly ($\tau = 1.18 \pm 0.17$ sec), while the levels of $PI(3,4)P_2$ and $PI(3,4,5)P_3$, indicated by PH(Akt), decreased slowly ($\tau = 1.36 \pm 0.07$ sec) and incompletely. The voltage dependent enzymatic activity of phosphatases was also investigated by addressing ramp depolarization which increases from 0 mV to 120 mV for 20 s (holding potential = −80 mV). PH(PLCδ) FRET started to decrease from the voltage of 33.0 ± 6.9 mV, whereas PH(Akt) FRET began to decrease from 71.0 ± 11 mV. In addition, recovery of $PI(4,5)P_2$ and $PI(3,4)P_2$ /$PI(3,4,5)P_3$ showed distinct phenomena in time constants (τ_{rev} = 7.17 ± 1.46 sec and 57.46 ± 4.78 sec, respectively) and in absolute quantity (92.3 ± 8.0 % and 55.3 ± 2.4 %, respectively). Thus, our results suggest that VSP can act as a dual phosphatase and deplete PIs with different time constants. The data also provide the molecular properties of endogenous PI resynthesis in living cells.

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Toward Understanding the Role of Amot130 Lipid Binding in Cellular Proliferation and Migration
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Background: Amots are adaptor proteins which coordinate signaling that controls cellular differentiation and proliferation. Amot proteins have a novel lipid binding domain, the Amot coiled-coil homology (ACCH) domain, which selectively binds monophosphorylated phosphatidylinositols (PI) and targets transcription factors to the nucleus. Understanding the biophysical mechanisms of lipid binding may provide pathways to modulate protein sorting and downstream signaling events inducing cellular differentiation, cancer cell proliferation, and migration. So far, all work reported on signaling based on Amot expression fails to distinguish between the role of the Amot80 and the 130 family members as they share a common ACCH domain.
Objective: The goal of this project is to specifically associate the Amot130 ACCH lipid binding with function related to ductal hyperplasia and breast cancer phenotypes.
Method: Mutations were carried forward based on lipid sedimentation, FRET, and SAXS assays against the ACCH domain. Site-directed mutagenesis was employed to probe the specific contributions of 7 selected lysines and arginines toward lipid head-group binding in the full length protein. Target proteins will be fluoresced to determine whether they retain their ability of binding to membrane. Cells fractionation will be used to quantify the protein amount that has passed the nuclear membrane.
Amot family members bind core polarity proteins controlling the apical domain organization of epithelial cells; and Yap, a transcriptional co-activator that regulates cell growth. Mutations in Amot affecting lipid binding to the apical membrane lead to disability to control cell growth and differentiation. Consequently, abnormal phenotypic changes regarding cell migration and polarization will be observed when growing cells on matrigel assays. Such mutations can also interfere with Amot binding to Yap, results in unregulated cell growth and proliferation. Yap expression level will be measured using western blots and immunoprecipitation techniques.

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Characterizing Pulmonary Surfactant Peptide and Lipid Interactions with Various Spectroscopic Techniques
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In vitro studies of pulmonary surfactant peptides in vesicles of mixed lipid composition are examined using nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) and fluorescence spectroscopy. As KL_4, a 21 residue mimetic of surfactant protein B (SP-B), has been used to restore lung compliance and promote gaseous exchange in premature babies with respiratory distress syndrome (RDS); and has served as a model in elucidating the mechanism by which lipids are trafficked to and from the air-fluid interface of alveoli. Here we utilize a pyrene phospholipid analog to investigate the effect of KL_4 on lipid organization and acyl chain dynamics by monitoring changes in excimer-to-monomer (I_e/I_m) ratio. This experiment probes the environment of the hydrophobic core of DPPC/POPG and POPC/POPG liposomes. An average decrease of ~27-40% and ~0-10% in I_e/I_m was observed in the DPPC/POPG and POPC/POPG LUVs, respectively, with increasing peptide concentration (0.5 to 5 mol%). This decrease is directly proportional to a lowered probability of excimer formation, which is highly dependent on proximal interactions of an excited monomer with a pyrene moiety at ground state. The ability of the peptide to modify membrane fluidity properties was studied via anisotropy measurements of a rhodamine-labeled phospholipid. A steady increase in the order was observed in the DPPC/POPG liposomes with relatively constant fluorescence intensity, while collisional quenching was observed in the POPC/POPG liposomes. Further studies were performed on the SP-B N and C-terminal constructs for correlation with NMR and EPR observations, and proposed mechanisms of peptide-mediated lipid trafficking.